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Riboflavin Requirement of Fingerling Red Hybrid Tilapia Grown in Seawater

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Abstract

Red hybrid tilapia *Oreochromis mossambicus* × *O. niloticus* fingerlings were fed diets containing 0, 2.5, 5.0, 10.0, 20.0 and 40.0 mg/kg, and 0, 2.5, 5.0, 7.5, 10.0 and 20.0 mg/kg of riboflavin in separate 8 and 12 wk feeding studies, respectively. The dietary riboflavin level required to provide maximum growth and survival, and prevent deficiency symptoms in red hybrid tilapia fingerlings was found to be approximately 5 mg/kg of diet. In both trials, fish fed the diet devoid of supplemental riboflavin exhibited anorexia, reduced growth and nervous symptoms after 4-6 wk. Mortality began to occur after the sixth week. None of these abnormalities were observed during the first 6 wk in fish fed the riboflavin supplemented diets. However, by the seventh week, fish fed the diet supplemented with 2.5 mg/kg of riboflavin showed reduced appetite and growth rate. In both experiments, short body dwarfism was observed during week 8 for fish fed the diet without riboflavin supplementation. In experiment 1, fish fed the riboflavin-deficient diet had lens cataracts at week 8. This deficiency sign was not observed in experiment 2. Histological studies of liver, kidney, spleen, lateral muscle, gill and gastrointestinal tract revealed no major histopathological changes.

Nutritional research for tilapia has not progressed as rapidly as with other species because tilapia culture has traditionally relied on natural pond food organisms to provide most or all of their nutritional needs. With this type of culture system, nutritionally complete diets were not necessary, thus, the establishment of basic dietary requirements for tilapia has not been a priority area of research. However, the increasing importance of tilapia in commercial aquaculture in both freshwater and seawater and the recent practice of intensive culture have created a need for research on the nutritional requirements of tilapia.

Riboflavin is an essential nutrient for all animals including fishes (NRC 1983). A number of riboflavin deficiency signs have been reported for various species of fish, but the only clinical indicators that are common among species are anorexia, poor growth and high mortality (Murai and Andrews 1978; Hughes et al. 1981a; NRC 1983). Established requirements for riboflavin are 4–10 mg/kg diet for common carp (Aoe et al. 1967; Ogino 1967; Takeuchi et al. 1980), 3–12 mg/kg diet for rainbow trout (Takeuchi et al. 1980; Hughes et al. 1981a; Woodward 1982, 1985; Amezaga and Knox 1990) 3–9 mg/kg diet for channel catfish (Murai and Andrews 1978) and 6 mg/kg diet for blue tilapia cultured in freshwater (Soliman and Wilson 1992). However, the quantitative riboflavin requirements for tilapia cultured in seawater have not been reported.

The objective of this study was to determine, through two separate feeding trials, the riboflavin requirements of fingerling red hybrid tilapia grown in seawater.

Materials and Methods

A series of two feeding experiments were conducted with red hybrid tilapia *Oreochromis mossambicus* × *O. niloticus* fingerlings obtained from the Mariculture Research and Training Center of the Hawaii Institute of Marine Biology, Kaneohe, Hawaii. The fish were acclimated for 2 wk from freshwater to seawater by gradual decrease of freshwater and increase of seawater, and held for another week in full strength seawater (32 ppt). During this period, they were fed with a commercial trout chow twice daily in excess. At the end of acclimation period, they were randomly selected and stocked in flow-through 55 L glass aquariums. Each aquarium, filled with 52 L of seawater, was covered with a plastic netting lid and provided with continuous aeration from an air blower. Water flow rate was checked and adjusted daily to insure proper water exchange rates. Photoperiod was maintained on a 12:12 h light : dark schedule.

All aquariums were cleaned daily by siphoning off accumulated waste materials. When fish were removed for weighing, the aquariums were cleaned thoroughly and drained. Water temperature, salinity, dissolved oxygen and pH were measured in three randomly selected aquariums three times per week.

TABLE 1. *Percentage composition and estimated nutrient content of basal diet.*

Ingredient and nutrient content	% In diet
Ingredient	
Casein (vitamin-free)	35.5
Gelatin	11.2
Dextrin	34.3
Corn oil	4.0
Cod liver oil	3.0
Mineral mix[a]	4.0
Vitamin mix (riboflavin-free)[b]	1.0
Carboxymethyl cellulose	3.0
Butylated hydroxytoluene	0.02
Cellufil	6.98
Estimated dietary nutrient (air dry weight)	
Crude protein (%)	38.0
Crude fat (%)	7.2
D.E. (channel catfish value) in kcal/kg diet	3,300

[a] Mineral mix: Williams and Briggs (1963) supplemented in mg/kg diet with cobalt chloride, 2.2; aluminum potassium phosphate, 12 hydrates, 5.0; sodium selenite, 0.2.

[b] Riboflavin-free vitamin mix (mg/kg diet): Thiamin hydrochloride, 10; pyridoxine hydrochloride, 10; calcium pantothenate, 40; niacin, 50; folic acid, 5; biotin, 2; vitamin B_{12}, 0.02; vitamin A, 4,000 IU; vitamin D_3, 2,000 IU; vitamin E, 50; menadione-Na-bisulfite, 10; inositol, 400; ascorbic acid, 300; choline chloride, 3,000.

The basal diet used in both experiments is presented in Table 1. The dry ingredients of each diet were mixed in a Hobart mixer, blended with 275 ml of water per kg of diet, and extruded through a 3/32 inch diameter die in a Hobart meat grinder. The resulting moist diets were cooled at room temperature, sealed in plastic bags and stored frozen at −40 C. Approximately 1 wk allowance of each diet was removed from the freezer, broken into small pieces and then held in a refrigerator (4 C) until fed.

In experiment 1, six purified diets containing 0, 2.5, 5.0, 10.0, 20.0 and 40.0 mg/kg of riboflavin were fed for 8 wk to triplicate groups of red hybrid tilapia fingerlings (initial mean weight of 1.46 ± 0.03 g) stocked at a rate of 25 fish/aquarium. Each diet was fed twice per day at a rate of 5% (wet weight) of biomass daily. Water flow rate was adjusted to obtain an exchange rate of 24 times per day. Water temperature ranged from 27.0 to 28.8 C with an average of 28.1 ± 0.3 C, salinity varied from 30.0 to 32.0 ppt with an average of 31.9 ± 0.4 ppt, and dissolved oxygen ranged from 5.3 to 8.8 ppm with an average of 7.6 ± 0.1 ppm. The pH values ranged from 7.4 to 7.8 with an average of 7.6 ± 0.1.

In experiment 2 which had the same objective as experiment 1, six diets were also used, but a diet supplemented with 7.5 mg/kg of riboflavin was added and the 40 mg/kg treatment was deleted. Each diet was fed for 12 wk to triplicate groups of red hybrid tilapia fingerlings (initial mean weight of 2.75 ± 0.04 g) stocked at a rate of 20 fish/aquarium. The fish were fed twice daily at a rate of 5% (wet weight) of their body weight per day for the first 6 wk. The rates were decreased to 4% and 3% for weeks 7–10 and 11–12, respectively. Water flow rates were

TABLE 2. *Average weight gain, survival, feed conversion and hematocrit of red hybrid tilapia fed various dietary levels of riboflavin for 8 wk (Experiment 1).*[1]

Dietary level of riboflavin (mg/kg)	Weight gain (g)	Survival (%)	Feed conversion[2]	Hematocrit (%)
0	5.77 ± 1.29^{a}	62.7 ± 16.2^{a}	1.38 ± 0.12^{a}	22.7 ± 2.6^{a}
2.5	11.45 ± 0.51^{b}	100.0 ± 0.0^{b}	0.82 ± 0.03^{b}	24.9 ± 1.3^{ab}
5.0	12.11 ± 0.86^{b}	100.0 ± 0.0^{b}	0.81 ± 0.05^{b}	28.2 ± 2.3^{ab}
10.0	12.37 ± 0.65^{b}	100.0 ± 0.0^{b}	0.79 ± 0.04^{b}	29.9 ± 0.8^{b}
20.0	12.17 ± 0.22^{b}	100.0 ± 0.0^{b}	0.80 ± 0.01^{b}	28.6 ± 2.7^{ab}
40.0	12.67 ± 0.06^{b}	100.0 ± 0.0^{b}	0.76 ± 0.03^{b}	28.1 ± 0.9^{ab}

[1] Values reported are means (± SD) of three replicates. Means in the same column having the same superscript are not significantly different ($P > 0.05$).

[2] Feed conversion = Dry feed fed (g)/Wet weight gain (g).

adjusted to obtain water exchange rates of 24, 32, 40 and 50 times per day for weeks 1–3, 4–6, 7–10 and 10–12, respectively. Water temperature ranged from 25.5 to 28.2 C with an average of 26.6 ± 0.7 C, salinity varied from 30.0 to 31.0 ppt with an average of 30.5 ± 0.3 ppt, and dissolved oxygen ranged from 5.3 to 8.3 ppm with an average of 6.3 ± 0.6 ppm. The pH values varied from 7.0 to 7.7 with an average of 7.4 ± 0.2.

In both experiments, fish in each aquarium were counted and weighed at 2 wk intervals. Daily feed allowances were adjusted every 2 wk based on sampling weights. On sampling day, fish were fed only once, in the afternoon, with 50% the amount of daily feed allowances. At the conclusion of both experiments, three fish from each treatment (one from each aquarium) were randomly selected for hematocrit determination using the microhematocrit technique of Blaxhall and Daisley (1973). Blood samples were obtained by amputation of caudal peduncle and drawing the blood directly into a microhematocrit tube. Three fish from each treatment were also collected for histological observations. Liver, spleen, kidney, heart, gill, skeletal muscle, integument, nervous system and intestinal tract were removed, fixed in 10% buffered formalin and decalcified using the formic acid A method (Humason 1979). Tissues were embedded in paraffin, sectioned at 4 μm thickness and stained with hematoxylin and eosin (Humason 1979). Radiographs of fish from treatments receiving 0 and 5 mg of supplemental riboflavin in experiment 1 were made at the end of the eighth week.

All data were subjected to the analysis of variance and Tukey's test to determine the differences between treatment means (Steele and Torrie 1960). Results were considered statistically significant at the 0.05 probability level.

Results

Experiment 1. The average weight gain, survival rate, feed conversion and hematocrit values are presented in Table 2. Anorexia and nervousness were observed after 3 wk for the group of fish fed the unsupplemented diet. By the end of 4 wk, the average weight gain of fish in this treatment was found to be significantly lower ($P < 0.05$) than the weight gain of fish in the other treatments. There were no significant differences among the weight gains of fish receiving riboflavin supplemented diets over the 8 wk period. However, a slight decrease in growth rate was observed between week 6 and 8 for fish fed the 2.5 mg riboflavin diet. Feed conversion ratios (g dry feed fed/g wet weight gain) were reflections of weight gains. Fish fed the diet without riboflavin supplementation had the poorest feed con-

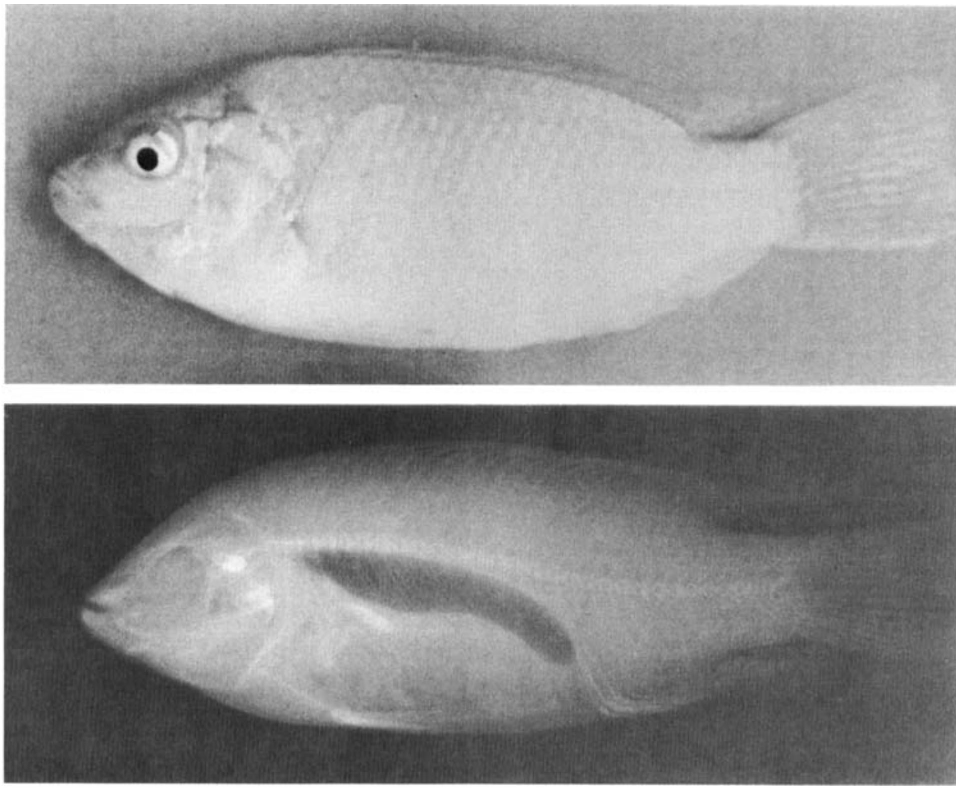

FIGURE 1. *Appearance and radiograph of normal red hybrid tilapia fed a diet containing 5 mg/kg of riboflavin for 8 wk.*

version. There were no differences among the feed conversion of the groups fed the other diets. Mortalities began to appear among the unsupplemented group during the sixth week. At the end of 8 wk, fish fed the riboflavin-deficient diet had a mortality rate of 37%, whereas those fed the vitamin supplemented diets had no mortality. Hematocrit values tended to be lower for fish fed diets containing 0 and 2.5 mg riboflavin as compared to those of the groups fed higher levels of dietary riboflavin.

Short body dwarfism was apparent by week 7 in the fish fed the unsupplemented diet, and at the end of week 8, 16–40% of the fish had incidence of short body. Radiographs (Figs. 1, 2) revealed that the dwarfism was due to shortening of individual vertebrae. Unilateral and bilateral cataracts were observed in 8–16% of fish fed the diet without riboflavin (Fig. 3). Histological observations indicated that lens opacity was primarily the results of hydropic swelling and lysis of the outer layers of the lens fibers, and occasional focal sites of epithelial hyperplasia, capsular reduplication and intralenticular migration of surface epithelium (Fig. 3B). These signs were not detected in any of the other groups of fish. No histological abnormalities were observed in the liver, spleen, kidney, heart, gill, skeletal muscle, integument, nervous system and intestinal tract of fish in any of the various treatments.

Experiment 2. Anorexia and neurological symptoms in fish fed the unsupplemented diet became apparent after the second week. By the end of 4 wk, the weight gain of this group was significantly lower ($P < 0.05$) than those of the other treatments (Table 3). The

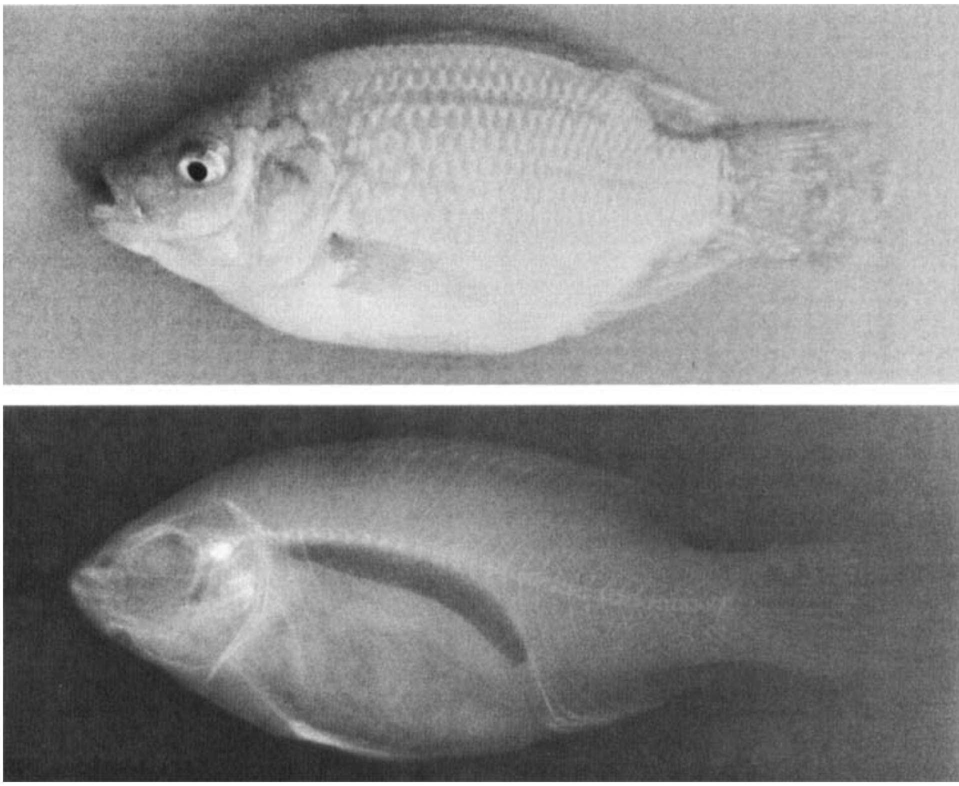

FIGURE 2. *Appearance and radiograph of red hybrid tilapia fed riboflavin-deficient diet for 8 wk showing short body and vertebrae.*

fish fed the diet containing 2.5 mg of supplemental riboflavin grew as well as the fish fed the higher levels of riboflavin for the first 6 wk as in experiment 1. By the end of 12 wk, the weight gain of this group of fish was significantly lower than that of fish fed diet supplemented with 5.0 mg riboflavin (Tables 3, 4). Higher levels of riboflavin supplementation did not result in further improvement in growth. Feed conversion values were similar for all riboflavin supplemented diets, and these were significantly lower than that of the non-supplemented diet.

The mortality rate of fish fed the diet without riboflavin supplementation was 63% as compared to 0 to 2% in the groups fed the supplemented diets (Table 4). Short body dwarfism was observed after 10 wk, and at the end of 12 wk 10–20% of the fish fed the unsupplemented diet developed short bodies (Figs. 1, 2). However, lens cataracts observed in experiment 1 were not detected. Histological study of various tissues revealed no apparent abnormalities.

Discussion

Results of this study indicate that red hybrid tilapia fingerlings fed riboflavin deficient diets had anorexia, poor growth, inefficient conversion of feed and high mortality. The same observations have been reported for chinook salmon (Halver 1957), common carp (Aoe et al. 1967; Ogino 1967; Takeuchi et al. 1980), channel catfish (Murai and Andrews 1978), rainbow trout (Takeuchi et al. 1980; Hughes et al. 1981a; Woodward 1982, 1984, 1985; Amezaga and Knox 1990) and blue tilapia (Soliman and Wilson 1992). Hemorrhages and dermatitis which have been reported in other studies with common carp (Aoe et al. 1967; Ogino

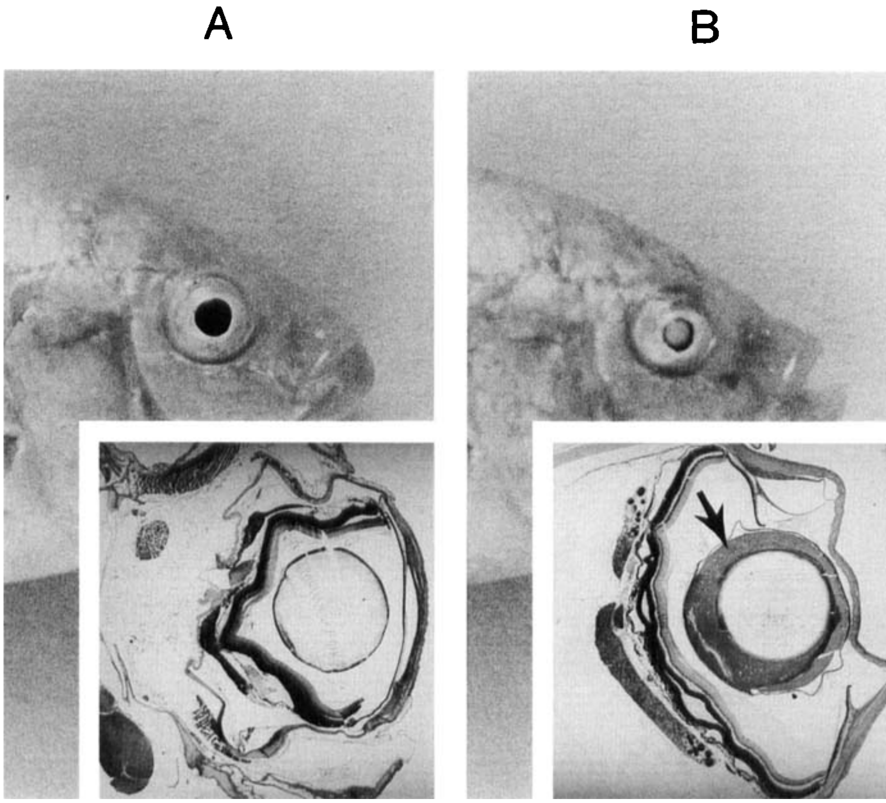

FIGURE 3. *Gross and microscopic appearance of eyes and lens of red hybrid tilapia fed diets with and without riboflavin supplementation for 8 wk. H&E. 10.85×. A. Normal eye and lens tissues of fish fed diet containing 2.5 mg/kg of riboflavin. B. Eye and lens tissues of fish fed riboflavin-deficient diet showing clouding of lens with hydropic swelling and lysis of the outer layers of the lens fibers.*

1967) and rainbow trout (Poston et al. 1977; Woodward 1984; Takeuchi et al. 1980; Amezaga and Knox 1990), and fin erosion and loss of normal body color reported in blue tilapia (Soliman and Wilson 1992) were not observed in the present study.

Short body dwarfism due to shortening of the individual vertebra found in fish fed the riboflavin-unsupplemented diet in the present study has also been reported in channel catfish (Murai and Andrews 1978) and blue tilapia (Soliman and Wilson 1992). Murai and Andrews (1978) indicated that this abnormal growth of spinal vertebrae may be related to hypothyroidism. They added, however, that there may be differences among fish species in riboflavin deficiency and hypothyroidism interactions.

Lens cataracts associated with riboflavin deficiency have been reported in salmonids (Halver 1957; Poston et al. 1977; Takeuchi et al. 1980; Hughes et al. 1981b; Amezaga and Knox 1990), channel catfish (Dupree 1966) and blue tilapia (Soliman and Wilson 1992). However, Woodward (1984) was not able to produce cataracts or corneal occlusion in rainbow trout fry and fingerlings, although the deficiency was sufficiently severe to cause high mortality. Likewise, Murai and Andrews (1978) did not observe eye lesions in channel catfish fed a riboflavin-deficient diet. In the present study, lens cataracts were detected only in fish fed the unsupplemented diet in experiment 1. The absence of lens cataracts in the experiment 2 may be attributed to the smaller size of

TABLE 3. *Cumulative weight gains at different periods for red hybrid tilapia fed various dietary levels of riboflavin for 12 wk (Experiment 2).*[1]

Dietary level of riboflavin (mg/kg)	Average weight gain per fish (g) at different periods (wk)					
	2	4	6	8	10	12
0	1.88 ± 0.03[a]	3.31 ± 0.38[a]	4.35 ± 0.50[a]	5.39 ± 0.51[a]	7.16 ± 0.61[a]	8.37 ± 1.30[a]
2.5	2.07 ± 0.09[a]	5.04 ± 0.13[b]	10.11 ± 0.49[b]	15.93 ± 1.52[b]	23.71 ± 2.84[b]	30.79 ± 4.74[b]
5.0	2.10 ± 0.09[a]	5.17 ± 0.18[b]	10.77 ± 0.26[b]	17.70 ± 0.42[b]	28.06 ± 0.35[b]	39.32 ± 0.36[c]
7.5	2.15 ± 0.19[a]	5.20 ± 0.27[b]	10.61 ± 0.45[b]	17.21 ± 0.10[b]	27.43 ± 0.97[b]	38.06 ± 2.44[bc]
10.0	2.14 ± 0.10[a]	5.18 ± 0.19[b]	10.79 ± 1.25[b]	17.90 ± 1.25[b]	27.70 ± 1.42[b]	38.64 ± 3.27[bc]
20.0	2.24 ± 0.32[a]	5.33 ± 0.38[b]	10.76 ± 0.90[b]	17.12 ± 0.80[b]	27.72 ± 0.80[b]	37.65 ± 1.21[bc]

[1] Values reported are means (± SD) of three replicates. Means having the same superscript are not significantly different ($P > 0.05$).

the fish in experiment 1, since, in both experiments, the same strain of fish and basal diet were used, and the water quality parameters and culture management were similar. This conclusion is supported by the fact that it took almost 20 wk for eye lesions to occur in 11.2 g rainbow trout in a study by Hughes et al. (1981b) as compared to only 11 wk in a study by Poston et al. (1977) in which 5.9 g fish were used. Corneal and lenticular abnormalities observed in the present study are typical of riboflavin deficiency as have been thoroughly described and characterized by Poston et al. (1977) and Hughes et al. (1981b) in rainbow trout. Hughes et al. (1981b) proposed that ocular lesions in rainbow trout are due to malfunction in the ribonucleic acid stages of protein metabolism in the unvascularized tissues, the lens and cornea.

There were no histopathological changes in other tissues attributed to riboflavin deficiency, except those of the eyes which have been described. This is in agreement with the findings of Murai and Andrews (1978) for channel catfish and Hughes et al. (1981b) for rainbow trout. Hughes et al. (1981b) indicated that histopathological signs of riboflavin deficiency are the last parts of the syndrome to be manifested.

Data from this study showed that a dietary riboflavin level of 5 mg/kg diet was adequate for maximum growth, feed efficiency and survival, and prevention of various deficiency signs in red hybrid tilapia fingerlings cultured in seawater. This value

TABLE 4. *Average weight gain, survival, feed conversion and hematocrit of red hybrid tilapia fed vairous dietary levels of riboflavin for 12 wk (Experiment 2).*[1]

Dietary level of riboflavin (mg/kg)	Weight gain (g)	Survival (%)	Feed conversion[2]	Hematocrit (%)
0	8.37 ± 1.30[a]	36.7 ± 15.3[a]	3.10 ± 0.40[a]	27.7 ± 3.2[a]
2.5	30.79 ± 4.74[b]	100.0 ± 0.0[b]	1.20 ± 0.13[b]	30.3 ± 2.5[a]
5.0	39.32 ± 0.36[c]	100.0 ± 0.0[b]	1.04 ± 0.00[b]	30.7 ± 2.5[a]
7.5	38.06 ± 2.44[bc]	100.0 ± 0.0[b]	1.07 ± 0.04[b]	28.3 ± 0.6[a]
10.0	36.64 ± 3.27[bc]	98.3 ± 2.9[b]	1.09 ± 0.06[b]	29.0 ± 1.0[a]
20.0	37.65 ± 1.21[bc]	100.0 ± 0.0[b]	1.07 ± 0.05[b]	30.7 ± 1.5[a]

[1] Values reported are means (± SD) of three replicates. Means having the same superscript are not significantly different ($P > 0.05$).

[2] Feed conversion = Dry feed fed (g)/Wet weight gain (g).

is in agreement with the ranges of the requirements reported for other fish species.

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